Neuron
Previews

Crucial First Steps: The Transcriptional Control of Neuron Delamination

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DOI 10.1016/j.neuron.2012.04.002

A crucial event in the birth of a neuron is the detachment of its apical process from the neuroepithelium. In this issue of *Neuron*, Rousso et al. (2012) show that repression of N-cadherin by Foxp transcription factors disrupts apical adherens junctions and triggers neurogenesis.

The neural stem and precursor cells (NPCs) that generate most of the neurons and glia in the mammalian nervous system are highly polarized. NPCs located in the neuroepithelium lining the ventricles of the neural tube extend a short apical process, which is attached to adjacent NPCs via adherens junctions. On their basal side, NPCs possess a longer process that contacts the pial basement membrane that surrounds the neural tube. When NPCs divide to generate new neurons, their daughter cells rapidly lose their apical attachment to the ventricular neuroepithelium, migrate away, and differentiate. The loss of apical process attachment is an important event during neurogenesis, which by itself is sufficient to initiate some of the subsequent steps in the neurogenic cascade. This is shown in experiments in which N-cadherin, an essential component of adherens junctions that maintains cell-cell adhesion via homophilic interactions, is experimentally eliminated. This manipulation results in the disruption of adherens junctions, the premature detachment of NPCs from the neuroepithelium, and the premature differentiation of the delaminated NPCs (Zhang et al., 2010). Elimination of other molecules associated with the apical junctions of NPCs, such as Cdc42, results in similar phenotypes (Cappello et al., 2006). By which mechanism newborn neurons detach their apical process from the ventricular surface at the onset of neurogenesis is therefore an interesting question, which has finally found an answer in the article by Rousso et al. (2012) in this issue of *Neuron*. The authors of this study demonstrate that two Forkhead transcription factors, Foxp2 and Foxp4, are essential to coordinate NPC delamination and differentiation during neurogenesis. Focusing on motor neuron development in the spinal cord of chick embryos, they show that misexpression of Foxp2 or Foxp4 results in the premature detachment of NPCs from the neuroepithelium and their differentiation into neurons. Consistently, the silencing of Foxp4, alone or together with Foxp2, produces the opposite phenotype: the detachment of NPCs is inhibited and the majority remains in an undifferentiated state, whereas differentiated cells are retained within the ventricular zone (VZ). Foxp2 and Foxp4 are known to be transcriptional repressors, and the authors show that direct repression of the N-cadherin gene is a key aspect in Foxp protein activity in the spinal cord. Misexpression of Foxp2 or Foxp4 results in a loss of N-cadherin expression in the VZ and a disruption of adherens junctions, whereas the combined knockdown of Foxp2 and Foxp4 has an opposite effect, causing an upregulation of N-cadherin mRNA and protein. Chromatin immunoprecipitation shows that Foxp4 binds to a regulatory element in the N-cadherin gene and therefore likely directly represses its transcription. Crucially, the authors provide compelling evidence that repression of N-cadherin is the key event that mediates the two activities of Foxp proteins in the spinal cord, i.e., their ability to promote both delamination and neuronal differentiation. First, high-level expression of a dominant-negative version of N-cadherin results in a disorganization of the neuroepithelium as well as in the premature differentiation of the delaminated cells, defects that are similar to those resulting from the misexpression of Foxp proteins. Second, expression of wild-type N-cadherin together with Foxp4 restores both the neuroepithelial architecture and the size of the progenitor pool, both of which are disrupted when Foxp4 is overexpressed alone. Together, these findings suggest that N-cadherin repression is the central signal by which Foxp proteins couple apical process detachment with the onset of neuronal differentiation in nascent spinal cord neurons.

The study by Rousso et al. (2012) together with earlier work from Matsumata and colleagues (Matsumata et al., 2005) suggest that the regulation of N-cadherin expression during neurogenesis might strongly influence the rate at which progenitors differentiate. Sox2 directly activates N-cadherin transcription (Matsumata et al., 2005) and therefore acts in opposition to Foxp4 to sustain N-cadherin expression levels and maintain the progenitor pool. Rousso et al. (2012) propose that the fine tuning of N-cadherin transcription by the combined input of Foxp4 and Sox2, and possibly other transcription factors, might determine the rate at which NPCs enter neurogenesis. Thus, the reduced level of N-cadherin in motor neuron progenitors compared to adjacent domains in the spinal cord may explain why motor neurons differentiate earlier than other populations of spinal cord neurons. However, the authors also provide evidence that Foxp proteins regulate neurogenesis by repressing target genes other than N-cadherin and in particular the Sox2 gene itself. Because Sox2 has been shown to inhibit neurogenesis by promoting N-cadherin expression (Matsumata et al., 2005) and antagonizing the activity of proneural transcription factors (Bylund et al., 2003), its repression might

also contribute significantly to the neurogenic activity of Foxp proteins.

The Foxp genes are expressed throughout the developing central nervous system, and Rousso et al. (2012) propose that their function in the cerebral cortex is broadly similar to that in the spinal cord. The cortex of Foxp4 mutant mice exhibits an increase in N-cadherin expression and a reduction in the number of differentiated neurons and, like in the spinal cord, some neurons remain in the progenitor zone. Conversely, Foxp4 overexpression in the mouse embryonic cortex by electroporation results in a downregulation of N-cadherin expression, a reduction in expression of Sox2, and a concomitant increase in expression of the intermediate progenitor marker Tbr2 (Rousso et al., 2012). This suggests that in the cortex as well as in the spinal cord, N-cadherin repression by Foxp4 overexpression results in a premature differentiation of VZ progenitor cells. These results are in general agreement with an earlier study by Zhang et al. (2010), who showed that N-cadherin knockdown in the embryonic cortex causes premature neuronal differentiation. An increased migration toward the developing cortical plate was, however, seen following N-cadherin silencing but not Foxp4 overexpression, and this discrepancy remains to be explained.

Overexpression of Foxp4 accelerates the differentiation of progenitors, suggesting that induction of this gene is an important step in the neurogenic program. Indeed, Rousso et al. (2012) provide evidence that Foxp4 expression is induced by the proneural transcription factor Neurogenin2 (Neurog2). However, unlike Neurog2, overexpression of Foxp4 is not sufficient to activate the whole neurogenic program. In particular, neurons prematurely induced by Foxp4 lose their attachment with progenitors but remain in the VZ, whereas neurons induced by Neurog2 overexpression migrate rapidly to the mantle zone (Mizuguchi et al., 2001). Therefore, factors other than Foxp proteins must promote the migration of newborn neurons downstream of proneural transcription factors. A possible candidate is the small GTP-binding protein Rnd2, which is induced by Neurog2 in newborn cortical neurons and promotes their migration via inhibition of RhoA signaling (Heng et al., 2008; Pacary et al., 2011) (Figure 1). Other factors acting downstream of Neurog2 in the developing cerebral cortex include the transcription factors insulinoma-associated 1 (Insm1) and Tbr2. Interestingly, like Foxp2 and Foxp4, Insm1 and Tbr2 promote the detachment of newborn neurons from the ventricular surface and their differentiation (Farkas et al., 2008; Sessa et al., 2008) (Figure 1). Future studies will hopefully determine whether these factors act by inducing Foxp proteins, by repressing N-cadherin themselves, or by other means of severing adherens junctions and promoting the delamination of newborn neurons.

The idea that the apical domain is required to sustain the self-renewal of NPCs, supported by the work of Rousso et al. (2012), has been challenged in recent years. Several studies examining the fate of the daughter cells of radial glial progenitor divisions in the cerebral cortex have concluded that cells that lose their apical process but retain a basal process can maintain a self-renewing progenitor state (Lui et al., 2011; Shitamukai et al., 2011). For example, in mice mutant for the G protein regulator LGN, the plane of neuroepithelial cell divisions is randomized, with the result that an increased fraction of progenitor cells lose their attachment to the ventricular surface and translocate to the intermediate zone. Strikingly, and unlike cells in which N-cadherin is disrupted, these cells continue to divide in their ectopic location (Konno et al., 2008; Shitamukai et al., 2011). Moreover, a novel type of self-renewing progenitor cells that have no contact with the ventricular surface, termed outer radial glial cells (oRGs), has recently been described in the cerebral cortex in several mammalian species, including mice, in which they are rare, and ferrets and humans, in which they are abundant (Fietz and Huttner, 2011; Lui et al., 2011). oRGs retain a basal process that may be important for the reception of signals maintaining the progenitor state, such as Notch signal. However, they are devoid of an apical process and apically located polarity molecules such as CD133, Par3, or aPKC (Fietz and Huttner, 2011; Lui et al., 2011). So, why do NPCs that express Foxp4 and lose their apical process attachment (but presumably retain a basal process) differentiate rather than continue to self renew? One possibility is that a neuronal fate determinant tethered to apical junctions in neuroepithelial NPCs is released by the disruption of adherens junctions and thus becomes free to promote differentiation (Bultje et al., 2009). Consistent with this model, Rousso et al. (2012) show that the Notch pathway inhibitor Numb is released into the cytoplasm when Foxp4 is overexpressed or N-cadherin activity is antagonized. They suggest that the resulting inhibition of Notch signaling might contribute to the initiation of neuronal differentiation that follows adherens junction disruption. In contrast, a change of plane of division, such as that occurring in LGN mutant mice (Konno et al., 2008), might segregate the daughter cell losing

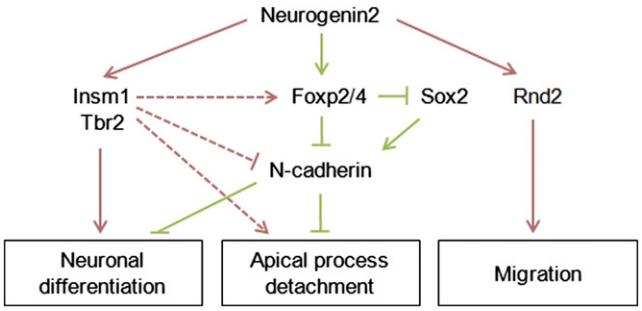

Figure 1. Model of Genetic Pathway Driving Neurogenesis
Rousso et al. (2012) show that Foxp2 and Foxp4 proteins function downstream of the proneural factor Neurogenin2 to promote both apical process detachment and neuronal differentiation. Foxp2 and Foxp4 inhibit N-cadherin by direct repression and, indirectly, by repressing Sox2, which promotes N-cadherin expression (green lines and arrows). Neurogenin2 also induces the transcription factors Insm1 and Tbr2, which like Foxp2/4 promote neuronal delamination and differentiation (Farkas et al., 2008; Sessa et al., 2008). Whether Insm1 and Tbr2 promote neuronal delamination by inducing Foxp2/4, by directly repressing N-cadherin, or by a distinct pathway is not known. Neurogenin2 also promotes neuronal migration by inducing the small GTP-binding protein Rnd2 (Heng et al., 2008). Lines and arrows in pink represent results obtained in the mouse cerebral cortex. Dashed lines and arrows indicate proposed but untested interactions.

the apical domain away from the apically localized neuronal fate determinant and thus allow this cell to remain proliferative. Further investigation should provide fascinating insights on how Foxp genes control the fate of neuroepithelial NPCs and contribute to the generation of other types of progenitors found in mammalian cortices.

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The CAP-Gly of p150: One Domain, Two Diseases, and a Function at the End

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DOI 10.1016/j.neuron.2012.04.003

In this issue of *Neuron*, work from Moughamian and Holzbaur (2012) and Lloyd et al. (2012) reveals a role for p150 in initiation of retrograde transport at synaptic terminals. These studies also suggest how mutations of p150's CAP-Gly domain lead to both Perry syndrome and HMN7B disease.

Although most cells are measured in microns, neurons, especially peripheral neurons, can be a meter long and therefore make extreme demands on our molecular motors. Small wonder that mutations in ubiquitous motor proteins give rise to specifically neurological diseases. Two such diseases, Perry syndrome and the distal hereditary motor neuropathy 7B (HMN7B), are examples of that phenomenon and their cell biological basis has been examined by two papers in this issue of *Neuron* (Moughamian and Holzbaur, 2012; Lloyd et al., 2012). Although their symptoms are quite different, both diseases are caused by mutations in the same domain of the dynactin subunit p150^{Glued}. By approaching the function of this domain in *Drosophila* neurons and mouse dorsal root ganglion (DRG) neurons, the present studies illuminate the function of p150^{Glued} in axonal transport.

Axonal microtubules are uniformly polarized with their plus ends away from the soma. Two classes of motor, kinesins and cytoplasmic dynein, move along these microtubule tracks to transport cargo between the soma and nerve terminals. Retrograde, minus-end-directed transport is performed by dynein. Two important functions of retrograde transport are escorting aggregated/misfolded proteins back to the soma for degradation (Johnston et al., 2002) and communicating synaptic and trophic signals to the soma to regulate gene expression (reviewed by Cosker et al., 2008). The dynein motors are multisubunit complexes, and much of the complex remains poorly understood. Moreover, dynein does not act alone; it acts in a complex with a second multimeric protein assembly known as dynactin. The largest subunit of dynactin is p150, the mammalian homolog of the *Drosophila Glued* gene (Holzbaur et al., 1991). Dynactin is mainly thought to be required for attaching cargo to dynein with p150 forming the dynein-dynactin link (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). Additional dynein-independent functions of p150 have been reported that involve organizing microtubule arrays and